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TITLE: **Pathways to Disease: The Biological Consequences of Social Adversity on Asthma in Minority Youth**

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14. ABSTRACT

Asthma incidence is increasing worldwide and disproportionately affects disadvantaged and minority populations. There is overrepresentation in the Active Duty military of low income and minority populations, including African Americans and Latinos. These populations experience the greatest social adversities and have significant asthma burden. The etiology of asthma-related disparities is multifactorial and known to be affected by poverty and its associated exposures. Chronic exposure to social adversities may trigger a stress response resulting in modulation of immune and hormonal responses and disruption of the body's microbiome. This toxic stress response is likely to be unique in each racial/ethnic group and depend on genetic susceptibility, the environment, and personal upbringing. The current proposal will address the cause, treatment, and prevention of asthma in high-risk populations. Aim 1 will focus on the immune system and hypothalamus-pituitary-adrenal axis response to social adversities and the effect on asthma outcomes (n=1000). Aim 2 will focus on the effect of social adversities on the microbiome and if the differences observed are associated with asthma (n=200). The proposal will allow for us to delineate the pathways by which social adversities impart their effects and identify points for intervention to improve asthma related outcomes.

15. SUBJECT TERMS
Nothing listed

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1. Introduction

Asthma incidence is increasing worldwide and disproportionately affects disadvantaged and minority populations. The etiology of asthma-related disparities is multifactorial and known to be affected by poverty and its associated exposures. There is overrepresentation in the Active Duty military of low income and minority populations, including African Americans and Latinos. These populations experience the greatest social adversities and have significant asthma burden, including higher asthma mortality. Chronic exposure to social adversities may trigger a toxic stress response resulting in modulation of the immune and hormonal response and disruption of the body's microbiome, both of which have been shown to negatively affect disease outcomes. This toxic stress response is likely to be unique in each racial/ethnic group and depend on genetic susceptibility, the environment, and personal upbringing. The current proposal will address the cause, treatment, and prevention of asthma in high-risk populations. This will be achieved by delineating the pathways by which social adversities impart their effects on asthma susceptibility and morbidity in minority populations. Aim 1 focused on the immune system and hypothalamus-pituitary-adrenal axis response to social adversities and their effect on asthma susceptibility and morbidity. We have measured 1000 of the proposed total of 1000 samples. Aim 2 will focus on the effect of social adversities on the microbiome and if the differences observed are associated with asthma. The measurement of the microbiome (n=200) has been completed. The proposal will allow for better identification of high-risk populations and development of interventions that target the modifiable aspects of social adversities to effectively improve asthma outcomes.

2. Keywords

Asthma, Adolescents, Young Adults, Chronic Stress, Socioeconomic Stress, Toxic Stress, Minority Health, Health Disparities, Protein-based Biomarkers, Microbiome, Allostatic Load.

3. Accomplishments

- **What were the major goals of the project?**

There are two major goals for the study that align with the Specific Aims. The **first major goal** is to measure biomarkers related to the immune and neuroendocrine system. We have measured immune- and neuroendocrine-related biomarkers on 1000 participants.

The **second major goal** is to measure and examine the oral microbiome in relation to measures of psychosocial and socioeconomic stress and asthma. Using PCR, we have amplified the V4 16S rRNA hypervariable region in 188 individuals.

- **What was accomplished under these goals?**

Under the **first major goal**, to measure biomarkers related to the immune and neuroendocrine system, we measured immune- and neuroendocrine-related biomarkers on 1000 participants. The biomarkers were measured in our laboratory using immunoassays or sent to our clinical laboratory.

As part of a preliminary study for this proposal, we measured TNF-alpha, a pro-inflammatory cytokine associated with both asthma and psychosocial stress, in our African American participants with asthma (n=576). As part of this proposal, we have completed an analysis

examining the effect of perceived racial discrimination on bronchodilator response (a measure of airway contractility) to albuterol (the mainstay rescue drug for asthma) among African American youth with asthma. We know that asthma is a multifactorial disease with varying risk profiles and outcomes, and thus, phenotypes. However, it is unknown which of these asthma phenotypes are vulnerable to psychosocial stress, the main exposure of interest for this proposal and a well described independent contributor to asthma morbidity. Almost half of participants (48.8%) reported experiencing racial discrimination. Those reporting discrimination were older (median age 15.4 versus 12.1 years, p<0.001), had a history of *in utero* smoke exposure (22.1 versus 15.3%, p=0.036), and had poorly controlled asthma (50.2 versus 33.9%; p<0.001). In the adjusted analysis, the mean BDR difference between participants reporting discrimination and those who did not was 1.70% (95%CI: 0.36-3.03%). However, this difference varied with TNF-α status (p=0.040). Among individuals with TNF-α high asthma, we observed a 2.78% greater mean BDR among those reporting perceived discrimination than those not reporting discrimination (95%CI: 0.79-4.77%). This difference was not seen in the TNF-α low asthma group (0.66%, 95%CI: -1.19-2.51%; **Table**). This is clinically important, as those who are at risk of poor asthma outcomes and were previously thought to be unresponsive to asthma medications may actually be responsive and may benefit from adjunct behavioral or environmental interventions. These results support screening for psychosocial stress in those with moderate-severe asthma as it may reclassify asthma type and delineate a treatment path. These results were presented as an oral abstract at the 2016 UCSF Health Disparities Forum (San Francisco, CA), the 2017 American Thoracic Society meeting (Washington, D.C.) and published in PLOS One as a scientific manuscript (Carlson PLOS One 2017).

Table: Mean Difference in Bronchodilator Response^ and 95% CI for Reports of Racial Discrimination and according to TNF-α status for SAGE II Participants with Asthma (2006-2014)

		TNF-α Status[2]	
	Adjusted[1]	**Low[1]**	**High[1]**
Racial Discrimination			
Never	Reference	Reference	Reference
Any	1.70 (0.36, 3.03)	0.78 (-1.07, 2.63)	2.78 (0.79, 4.77)

^ Bronchodilator response: mean percentage change in measured FEV_1 before and after albuterol administration, using the post-albuterol spirometry with the maximal change.
[1]adjusted for sex, age, maternal education, recruitment center, *in utero* smoke exposure, daycare attendance, baseline lung function, controller medication use, African ancestry, TNF-α mean, and biomarker storage time.
[2]p-interaction = 0.04

Among the 1000 participants with measured biomarkers, 689 had complete data for socioeconomic status, experiences of discrimination, and early-life NO_2 exposure (a marker for traffic-related air pollution). These three variables represent different aspects of adversity: socioeconomic, psychosocial, and environmental stress. From these three exposure variables, a composite adversity variable was developed. Participants with 2 or more exposures were consider to have high adversity exposure and those with 1 or less exposure were considered to have low adversity exposure. CCL17 (thymus and activation regulated **chemokine**- TARC) is produced in the thymus by dendritic cells and binds to a region on Th2 lymphocytes and induce an allergic response (elevated in Th2 high asthma— a well-defined atopic endotype of asthma). Among children without asthma, participants with the high adversity were more likely to have elevated CCL17/TARC compared with those with low adversity (94.0 pg vs 71.8 pg,

p=0.06); no difference in CCL17/TARC levels were noted among kids with asthma by adversity exposure. Similarly, we found that CHI3L1 (YKL-40), a cytokine elevated in asthma, was elevated in healthy controls with adversity exposure in comparison with children without adversity p=0.03) and no difference was observed among children with asthma. These findings suggest that adversity exposures are associated with increased atopy response in children without asthma. We may not observe a similar response in children with asthma due to an overall increase due to the underlying disease state versus a differential based on case/control status. Our findings will be further explored by examining other cytokines by adversity exposure and examining skin-prick testing to evaluate for allergen sensitization status by adversity exposure (gold standard for confirming positive atopy response).

The **second major goal** is to measure and examine the oral microbiome in relation to measures of psychosocial and socioeconomic stress and asthma. We deep sequence the 16s rRNA gene from the DNA in 188 saliva samples from participants. DNA was extracted on all samples and the V4 16S rRNA hypervariable region was amplified. Samples were sequenced by pair-end 300 base pair reads in a MiSeq sequencer. We will now compare the measured V4 region from our samples to recorded libraries to define the oral microbiome in terms of richness, diversity and bacterial taxonomy. This will then be examined as it relates to stress exposures and asthma.

- **What opportunities for training and professional development has the project provided?** *Nothing to Report*

- **How were the results disseminated to communities of interest?**

The participants included in this study are from clinics that serve predominantly under-insured minority communities that experience an excess of social adversities and chronic stress. The results of the study examining the role of perceived discrimination on drug response among African American youth with low and high TNF-alpha were shared with clinical providers and outreach coordinators from the Center of Youth Wellness (Bayview neighborhood, San Francisco, CA) and UCSF Children's Hospital Oakland (Oakland, CA) in the format of a journal club. We were able to discuss the potential clinical implications and the necessary next steps to confirm our findings.

- **What do you plan to do during the next reporting period to accomplish the goals?**

For **project goal 1**, we have completed measurement of the inflammatory and neuroendocrine biomarkers on 1000 participants. We are 1) examining how the biomarkers differ by asthma diagnosis (case/control study), and 2) determining if these biomarkers differ by stress exposure in those with and without asthma (stratified analysis). These analyses will be completed in the next six months.

For **project goal 2** we have measured the oral microbiome by sequencing the 16S rRNA region. Currently, we are performing quality control. We will then define the diversity, richness, and abundance of the micrbiome in the next 6 months.

4. Impact

- **What was the impact on the development of the principal discipline(s) of the project?**

The preliminary results, the content expertise, and the infrastructure developed from this proposal led to the successful funding for a longitudinal study of early-life exposure to adversity and health, including asthma. The TARA Health Foundation awarded $4.8 Million dollars to establish the Bay Area Research Consortium on Toxic Stress and Health; UCSF (Thakur) received $819,415 to examine biomarkers as they relate to social adversity, stress, and health (study period: 2015-2019). This study comprehensively measures exposure to trauma and adversity in childhood that are commonly associated with post-traumatic stress disorder in adulthood and will follow the enrolled children longitudinally. We are obtaining biospecimens at several time points over the course of the study and measure inflammatory and neuro-endocrine biomarkers, the microbiome, and teleomere length and relate these biomarkers to the measured exposures to adversity and stress. The selection of and methods to measure the biomarkers were directly informed by this study and represent the natural next step from the current study.

In 2016, the Koret Foundation awarded University of California, Berkeley to establish the Koret Institute of Precision Prevention (KIPP) Center. As part of this center, UCSF was awarded a subcontract (PI: Thakur, $331,885) to implement in health study in Richmond, CA to examine the effect of social and environmental stressors based on asthma type among adolescents with and without asthma. This study will mirror the above study, and, together, these studies have the opportunity to change the way we think about social and environmental adversities and health by providing a biological framework and identifying critical points for intervention.

- **What was the impact on other disciplines?** *Nothing to Report*
- **What was the impact on technology transfer?** *Nothing to Report.*
- **What was the impact on society beyond science and technology?**

The results of this study have the potential to have great impact on how we classify asthma and determine treatment path. The results from our TNF-alpha and discrimination and our preliminary results suggest that these social stressors effect different pathways and may provide insight on how to approach those with moderate to severe asthma. We may find that those who are at risk of poor asthma outcomes and previously thought to be unresponsive to asthma medications may actually be responsive and may benefit from adjunct behavioral or environmental interventions.

5. Changes/Problems

- **Changes in approach and reasons for change:** *Nothing to Report.*
- **Actual or anticipated problems or delays and actions or plans to resolve them**

The proposed timeline for our project was delayed. We experienced an initial delay of 3 months while the Department of Defense's Human Research Protection Office completed their review of the project. This review resulted in a local (UCSF) Institutional Review Board Amendment of the project and we were granted approval from the HRPO at the end of December 2015. After selecting a subset of our study population for evaluation, we experienced a second delay of almost three months in setting up our account for Clinical lab testing. At this time, we have measured the proposed biomarkers and microbiome and are currently in the analytical stage of the proposal.

- **Changes that had a significant impact on expenditures**

While there have not been any significant changes in the overall cost of the project, the delays listed above have shifted the overall timeline of expenditures by six months.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:** *Nothing to Report*
- **Significant changes in use or care of human subjects:** *Nothing to Report*
- **Significant changes in use or care of vertebrate animals** N/A
- **Significant changes in use of biohazards and/or select agents** N/A

6. Products:

- **Publications, conference papers, and presentations:**

  S Carlson, LN Borrel, SS Oh, C Eng, A Davis, K Meade, HJ Farber, E Brigino-Buenaventura, S Thyne, S Sen, MA LeNoir, N Burke-Harris, EG Burchard, N Thakur. Perceived Discrimination Affects Bronchodilator Response in African American Youth with Asthma. UCSF Health Disparities Research Symposium 2016. San Francisco, CA October 2016. (Accepted for Oral Presentation)

  N Thakur, S Carlson, LN Borrel, SS Oh, C Eng, A Davis, K Meade, HJ Farber, E Brigino-Buenaventura, S Thyne, S Sen, MA LeNoir, N Burke-Harris, EG Burchard. Perceived Discrimination Affects Bronchodilator Response in African American Youth with Asthma. 2017 American Thoracic Society. Washington, D.C. May 2017. (Accepted for Oral Presentation)

- **Journal publications.**

  S. Carlson, Borrell N, Eng C, Nguyen M, Thyne S, LeNoir MA, Burke-Harris N, Burchard EG*, Thakur N*. *These authors contributed equally to this work. Self-reported racial/ethnic discrimination and bronchodilator response in African American youth with asthma. PLoS ONE 12(6): e0179091. PMID 28609485

- **Books or other non-periodical, one-time publications:** *Nothing to Report*
- **Other publications, conference papers, and presentations.**
- **Website(s) or other Internet site(s):** *Nothing to Report*
- **Technologies or techniques:** *Nothing to Report*
- **Inventions, patent applications, and/or licenses:** *Nothing to Report*
- **Other Products**

Database: With this study, we have added plasma-based biomarker measurements and microbiome data to the SAGE II and GALA II datasets. These two pieces of the information will

allow it to be possible to perform analyses across multiple levels of data ranging from the plasma-based biomarkers to environmental data.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	Neeta Thakur
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-6126-6601
Nearest person month worked:	3
Contribution to Project:	Dr. Thakur oversaw the measurement of biomarkers, came up with the research question and analytical plans for the preliminary study.
Funding Support:	NHLBI K23 Career Development Award, Parker B. Francis Fellowship Program, TARA Health
Name:	Sam Oh
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	0000-0002-2815-6037
Nearest person month worked:	1
Contribution to Project:	Dr. Oh
Funding Support:	NIH/ NIMHD; NIH/NHLBI; NIH/NIEHS; DOD; Harvard Pilgrim Health Care, Inc.
Name:	Celeste Eng
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Ms. Eng
Funding Support:	INO Therapeutics; NIH/NIMHD; NIH/NHLBI: University of California Tobacco Related Disease Program, Tara Foundation *
Name:	DongLei Hu
Project Role:	Biostatistician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Hu

Funding Support:	INO Therapeutics; NIH/NHLBI; NIH/NCI; DOD; City of Hope/NIH/NCI; NIH/NIMHD; Harvard Pilgrim Health Care, Inc.
Name:	Sonia Carlson
Project Role:	Medical Student
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	4
Contribution to Project:	Ms. Carlson
Funding Support:	NIH/NIMHD UCSF PROF-PATH

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** No Change

- **What other organizations were involved as partners?**

Organization Name: Center for Youth Wellness

Location of Organization: *San Francisco, CA*

Partner's contribution to the project

In-kind support: SAGEII and GALAII include a total of 6,500 participants. This proposal allows for the measurement of biomarkers in 1000 of these participants. The CYW provided assays and associated materials for the measurement of biomarkers in an additional 750 individuals from the SAGEII study.

8. Special Reporting Requirements: NA

9. Appendix: NA